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Spontaneous polymerization of the antibiotic peptide magainin 2

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We describe here the ability of the magainin 2 peptide to assemble spontaneously into characteristic 13-nm diameter filaments having a 30 nm periodic helical substructure. Optimal conditions for extensive polymerization into filaments of several hundred microns required low pH and high ionic strength. Polymerization of the magainin 2 peptide may be involved in its recently described in vitro membrane-disrupting and antibiotic activities.

Polymerization; Antibiotic mechanism; Electron microscopy; Magainin 2

1. INTRODUCTION

A family of peptides shown to have antimicrobial activity in vitro against a broad range of microorganisms including fungi, protozoa, gram-positive and gram-negative bacteria, has been recently isolated from the skin of the African clawed frog *Xenopus laevis* [1–3]. This class of peptides, named magainins, are secreted by the granular gland of the amphibian skin [4]. The two most active forms are magainin 1 and magainin 2 (MAG 2), differing in two substitutions [1]. Magainins may represent a newly discovered natural defense mechanism in vertebrate organisms, complementary to the cecropins described in invertebrates. Their peptide structure offers new possibilities for antimicrobial drug design [2].

The antimicrobial activity of the peptides is presumed to depend on interactions with biomembranes [5–7]. MAG 2 has been shown to interact with a synthetic lipid bilayer to form anion-selective channels [6]. The channel formation is postulated to involve an assembly of a variable number of MAG 2 molecules (Cruciani et al., in preparation). Here, we describe conditions under which MAG 2 molecules spontaneously form characteristic linear polymeric structures. Such an ability to form polymer aggregates may be involved in the channel formation and antimicrobial activity displayed by this peptide.

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2. EXPERIMENTAL

Synthetic MAG 2 purified to homogeneity by reverse-phase high-pressure liquid chromatography [2,3] was a gift from Dr M. Zasloff. MAG 2 (20 mg/ml) was dissolved in solutions ranging from 0.1 to 1 M NaCl. Potassium acetate (10 mM) was used for pH adjustments between 4 and 6 and Tris-HCl (10 mM) for pH between 6 and 8. MAG 2 (20 mg/ml) dissolved in distilled water had a pH of approx. 3. Filament formation was monitored by both light and electron microscopic techniques after incubations at either 4, 25 or 37°C.

2.1. *Light microscopy*

MAG 2 filament bundles were visualized using a Zeiss Axiomat microscope in polarization and differential interference contrast mode equipped with an internally corrected × 100, 1.3 NA, planapochromatic objective. For video-enhanced light microscopy, the aperture of a 1.4 NA condenser was fully illuminated with a 100 W mercury lamp aligned for critical illumination [8] and the optical image was projected out of the camera port of the microscope onto a Newvicon Dage-MTI 68 video camera (Dage-MTI, Michigan City, IN).

2.2. *Electron microscopy*

For rotary shadowing, solutions of polymerized MAG 2 were made in 50% glycerol, sprayed onto a freshly cleaved piece of

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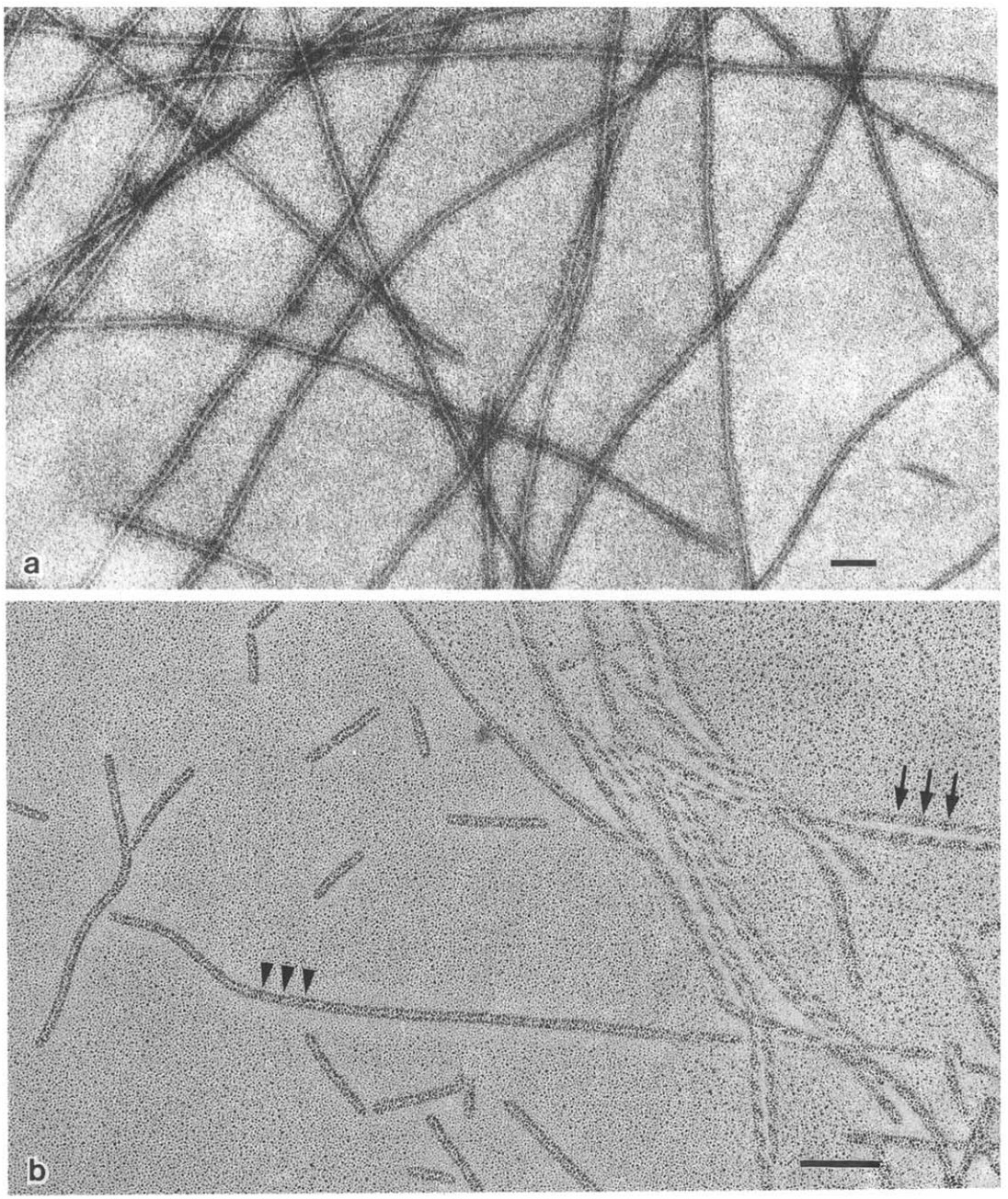

Fig.1. Electron microscopy of polymer filaments obtained after incubation of 20 mg/ml of magainin in 0.1 M NaCl for 48 h at room temperature. The helical substructure of the filaments is not visualized in negative staining (a) but is apparent in replicas of rotary shadowed preparations (b). Within each drop of the polymerized magainin solution sprayed onto the mica and dried prior to rotary shadowing, the helical pattern is less evident in the periphery (arrowheads) than in the center (arrows). This is due possibly to the accumulation of salt as the drop progressively dries under vacuum which can be visualized as an increase in the granularity of the background as one goes towards the center of the drop (from the left to the right of b). Bar, 100 nm.

mica, placed under 10^{-6} Torr vacuum for 5 h in a Balzers 301 freeze-etching unit and rotary shadowed with platinum/carbon [9]. For negative staining we used 10-μl drops of MAG 2 solutions which were placed on the surface of glow-discharged formvar carbon-coated grids. After 30 s the grids were blotted and stained for 30 s in 1% uranyl acetate. Micrographs were taken with a Jeol 100 CX electron microscope.

3. RESULTS

Under physiological conditions, MAG 2 is a very

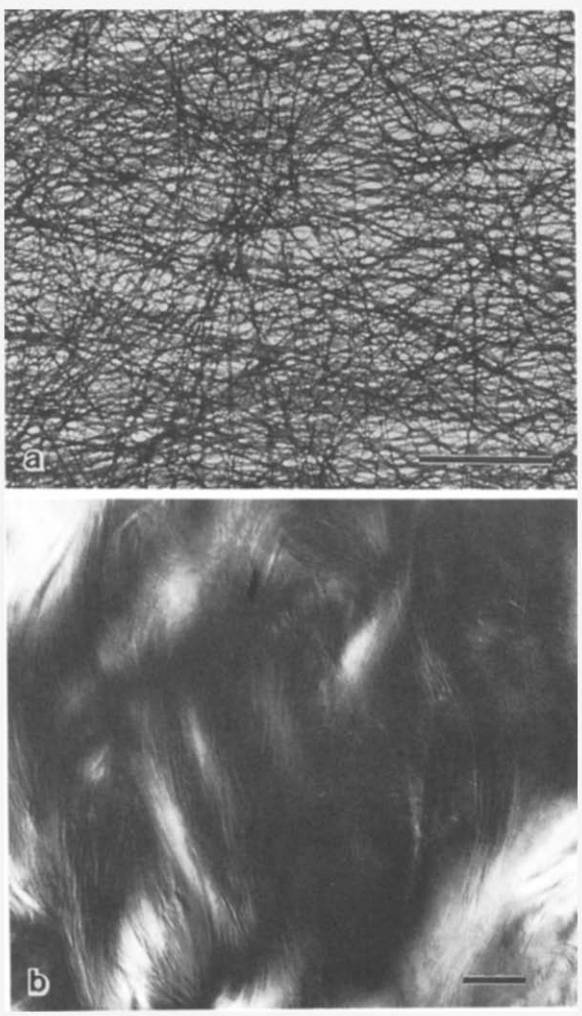

Fig.2. Massive polymerization of MAG 2 after 24 h incubation in 1 M NaCl visualized with negative staining (a) and polarized light microscopy (b). Birefringence becomes evident when fiber bundles change brightness as the specimen is rotated between the cross polarizers. Bar, 1 μm.

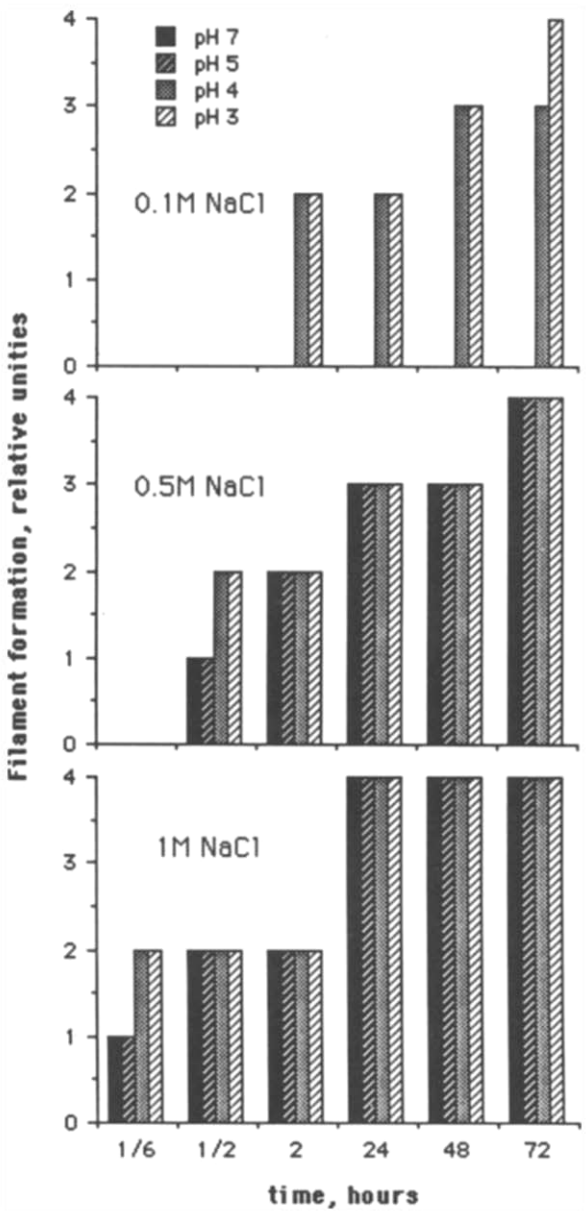

Fig.3. NaCl concentration and pH dependence of the MAG 2 polymerization reaction. Filament formation was monitored with light and electron microscopy. Relative values were arbitrarily defined as 0, when no filaments were observed by negative staining; 1, when the density in the negative staining averaged between 0 and 5 filaments per μm; 2, when density averaged between 5 and 50 filaments per μm; 3, when the density of filament was more than 50 filaments per μm and occasional filament bundles could be seen with the light microscopy; 4, when the solution becomes extremely viscous and a dense amount of birefringent filament bundles are seen with the light microscope.

soluble polypeptide. When the pH is lowered and the salt concentration is increased, MAG 2 self-assembles into filaments several micrometers long as shown in fig.1a. The diameter of these filaments measures 12.8 ± 0.7 nm in the negative staining preparations and 12.8 ± 1.1 nm in the rotary shadowed replicas. Rotary shadow preparations of these filaments also revealed a helical substructure with a periodic 30.2 ± 1.4 nm pitch (fig.1b). The smallest polymer structures that we characterized with the electron microscopy were in the form of short filaments. It is not clear how the polymerization is initiated, since the resolution limit of both negative staining and rotary shadowing is not sufficient to resolve small aggregates of the MAG 2 polypeptide which has only 23 amino acids. Polymerization proceeded in 1 M NaCl, and over the course of 24 h the solutions became very viscous with massive amounts of filaments hundreds of micrometers long (fig.2a). These filaments associated with each other to form birefringent bundles which could be visualized directly in a light microscope under polarized light (fig.2b). The filament bundles could also be visualized by video-enhanced differential interference contrast microscopy (not shown). Using both video microscopy and negative staining we established an arbitrary scale (see fig.3) to study the salt, pH and temperature dependence of the polymerization reaction of MAG 2.

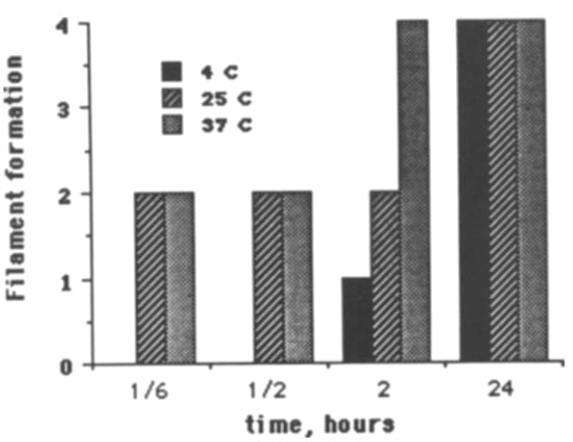

Fig.4. Temperature dependence of MAG 2 self-assembly. MAG 2 (20 mg/ml) solutions were prepared in 1 M NaCl. Filament formation was measured using the same arbitrary values described for fig.3.

At 100 mM NaCl filament polymers were observed only below pH 5 (fig.3). At 500 mM NaCl, filaments were detected at pH 7 after 30 min incubation and at 1 M NaCl filaments were visualized as early as 10 min of incubation. The rate of filament formation increased as the temperature was raised from 4 to 37°C (fig.4).

4. DISCUSSION

We show here that MAG 2 is capable of generating defined polymer structures. Rapid polymerization into characteristic 13-nm wide filaments required lowering the pH and raising the salt concentration. Large hydrophobic moments can be predicted from the amino acid composition of MAG 2 [1]. Lowering the pH and increasing the salt concentration and the temperature are all conditions that would enhance the hydrophobicity of MAG 2 [10]. The spontaneous polymerization of MAG 2 under such conditions may therefore result from hydrophobic interactions between the MAG 2 molecules.

From experiments using two-dimensional NMR spectroscopy, Marion et al. [7] suggested that MAG 2 monomers might self-associate while surrounded by a hydrophobic environment. They have also shown that the peptide typically changes from a random coil conformation in aqueous solutions to an α-helix in hydrophobic solvents. Thus, formation and stabilization of the filament polymers may also depend upon the transition to an amphiphilic α-helix in each monomer.

The amphiphilic α-helix configuration of magainin has been shown to be important for its biological activity. Increasing the helix content, which can be induced with hydrophobic solvents such as trifluoroethanol, also increases the antibiotic activity [5,7]. It is also postulated that the amphiphilic property of MAG 2 permits its interaction with biological membranes or artificial lipid bilayers [5–7]. In fact, it has recently been reported that MAG 2 increases the conductance of artificial lipid bilayers [6] through the formation of anion-selective channels [6]. These channels are postulated to be formed by the assembly of a number of MAG 2 molecules. Electrophysiological data have also suggested that alamethicin, a peptide synthesized by fungi, aggregates in six or more

molecules per channel in artificial lipid bilayers [11].

It is possible that the ability of MAG 2 to polymerize in solution under conditions of increased hydrophobicity is related to its ability to aggregate within bilayers and to form membrane channels. In addition, the formation of characteristic filament structures reported here may provide a basis for further knowledge about the native role of this secretory product. For example, it is possible that magainins are involved in forming some extracellular structural components or a filamentous surface coat in epithelial tissues of the frog.

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